B-cell responses in allergen immunotherapy

Satitsuksanoa, Pattraporn ; van de Veen, Willem ; Akdis, Mübeccel

Abstract: URPOSE OF REVIEW: The establishment of long-term clinical tolerance in AIT requires the involvement of basophils, mast cells, allergen-specific regulatory T and B cells, downregulation of effector type 2 responses, and increase in production of specific IgG, particularly immunglobulin G4 (IgG4) antibodies. This review aims to provide an overview of the role of B cells in AIT, their mechanism of action, and their potential for improving AIT. RECENT FINDINGS: In-depth research of B cells has paved the way for improved diagnosis and research on allergic diseases. B cells play a central role in allergy and allergen tolerance through the production of immunglobulin E (IgE)-blocking antibodies. However, an increasing body of evidence has emerged supporting a role for B cells in regulating immune responses that extends beyond the production of antibodies. Regulatory B cells play an important role in immunosuppression, mediated by secretion of anti-inflammatory cytokines. SUMMARY: Successful AIT establishes the reinstatement of immune tolerance toward allergens, reduces allergic symptoms, and improves clinical treatments in patients. B cells play a central role in this process through antibody-independent immune regulatory processes in addition to the production of IgE-blocking antibodies.

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B-cell responses in allergen immunotherapy

Pattraporn Satitsuksanoa[a,b], *Willem van de Veen*[a,b], *and Mübeccel Akdis*[a,b]

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Successful AIT establishes the reinstatement of immune tolerance toward allergens, reduces allergic symptoms, and improves clinical treatments in patients. B cells play a central role in this process through antibody-independent immune regulatory processes in addition to the production of IgE-blocking antibodies.

Keywords
allergen-specific immunotherapy, anti-inflammatory cytokines, IgE, IgG4, immune tolerance, regulatory B cells

INTRODUCTION

Allergic diseases remain a major global health problem causing significant morbidity and mortality and accounting for a considerable portion of healthcare expenditures [1]. Allergic diseases develop from many heterogeneous diseases with different clinical manifestations. These diseases generally result from an uncontrolled inflammatory response to allergens and can lead to a number of disorders, including food allergy, asthma, allergic rhinoconjunctivitis, atopic dermatitis, and anaphylaxis [2]. Food allergy is affecting up to 8% of children and 5% of adults in westernized countries and, development of therapies for this potentially life-threatening condition has become a public health priority [3]. Allergic rhinoconjunctivitis is an allergic disorder of the nose and eyes affecting about a fifth of the general population [4]. The prevalence of atopic dermatitis in longitudinal birth cohort studies is similar in childhood and adolescence/early adulthood [5]. Anaphylaxis occurs with an incidence of 1.5–7.9 per 100 000 people/year [6].

There are different endotypes in allergic disorders, which are defined by the compilation of disease mechanisms explaining disease expression in groups of patients. The most common endotypes for allergic diseases are type 2 and nontype 2; however, recent data have emerged in support of inflammasome subtype, barrier subtype, type 17 subtype, and mixed types such as type 2/type 1 and type 2/type 17 as well as allergen-specific immunotherapy (AIT)-responsive endotype [1].

For example, the endotypes in food allergy consists of immunglobulin E (IgE)-mediated immediate hypersensitivity reactions, non-IgE-mediated reactions, and disorders with mixed IgE-mediated and cell-mediated immune reactions [7▪▪]. IgE-mediated allergies are typically immediate onset with reactions ranging from mild to severe to life-threatening anaphylactic reactions involving single or multiple organs. Non-IgE and mixed endotypes could be shown in food protein-induced enterocolitis syndrome (FPIES) and eosinophilic esophagitis (EoE). FPIES, diagnosed in infants and toddlers (with

[a]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich and [b]Christine Kühne-Center for Allergy Research and Education (CK-CARE), Davos, Switzerland

Correspondence to Mübeccel Akdis, MD, PhD, Swiss Institute of Allergy and Asthma Research (SIAF) Herman-Burchard-Strasse 9, CH- 7265 Davos, Switzerland. Tel: +41 81 4100848; fax: +41 81 4100840; e-mail: akdism@siaf.uzh.ch

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KEY POINTS

- AIT induces clinical immune tolerance by reducing symptoms of allergic patients and has been used as a possible curative treatment for allergies for more than a century.
- Breg cells play an important role in induction and maintenance of immune tolerance during AIT through IL-10-mediated suppression of effector T cells, inhibition of dendritic cell maturation, induction of Treg cells and production of antiinflammatory IgG4 antibodies.
- Recently introduced knowledge on the role of B cells in AIT are beneficial for vaccine development and may lead to improved AIT strategies for the treatment of allergic patients.

spontaneous resolution within 1–5 years), includes reactions with vomiting and diarrhea primarily because of food allergy to cow's milk or soy's milk [8]. Patients with EoE have increased levels of thymic stromal lymphopoietin, whereas patients with EoE-like disease can be distinguished from EoE by eotaxin-3, Mucin 4, and cadherin-like protein 26 expression levels [9]. Recently, it was shown that desmoglein-1, an intracellular adhesion molecule, is downregulated by IL-13 leading to impaired barrier function. Tracking of desmoglein-1 in individuals with EoE can aid in diagnosis and management, leading to improved precision medicine [10]. Recent studies have shown that children with EoE have high or very high titers of immunglobulin G4 (IgG4) and low but detectable IgE [11].

One of the most successful treatments that are available for allergic patients is AIT [12–14]. AIT is an immune tolerance-inducing treatment that reduces symptoms of allergic rhinitis and asthma as well as venom allergy and food allergy [15▪▪,16–18]. It involves the administration of increasing doses of the causative allergen and induces the establishment of long-term clinical tolerance against allergens [19–21]. Routes of AIT consist of subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT). SCIT is used as a reference therapy and has transformed allergic treatments; it improves symptoms (asthma and rhinitis) as well as the quality of life of patients [22,23]. SLIT is now a valid noninvasive alternative to SCIT, as a well-tolerated and efficacious treatment for respiratory allergies [24–27].

The molecular and cellular mechanisms of allergen tolerance in humans have been intensively studied during the past decades, leading to the identification of crucial processes in the induction and maintenance of allergen tolerance. These include induction of allergen-specific regulatory subsets of T and B cells, secretion of immune suppressive secreted factors such as IL-10 and transforming growth factor-β (TGF-β), production of allergen-specific IgG4 blocking antibodies, and a decrease in allergic inflammatory responses by mast cells, basophils, and eosinophils in inflamed tissues [28,29]. It has been known that regulatory T cells play a central role in the induction and maintenance of allergen tolerance [30]; however, data supporting a role for B cells in these processes have been increasing in recent years. In this review, we discuss the role of B cells in allergen-specific immunotherapy (AIT).

MECHANISM OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

Common allergens originate from naturally occurring proteins of plant and animal-origin [31]. The allergens are initially broken down by hydrolytic enzymes in the gastrointestinal tract during the digestive process. It is hypothesized that allergens can be modified into different forms and different structures, which can be processed by antigen-presenting cells, presented on the major histocompatibility complex class II molecules and subsequently recognized by antigen-specific T cells. The naïve antigen-specific T helper cells differentiate into effector T-helper 2 (Th2) cells in the presence of IL-4. A set of interleukins such as IL-4, IL-5, IL-10, and IL-13 are produced by Th2 cells and induce B cells to differentiate into IgE-producing plasma cells. Antigen-specific IgE antibodies directly bind to high-affinity receptor FcεRI on mast cells and basophils. Upon reexposure to the allergen, these specific IgE antibodies induce degranulation of mast cells and release of mediators including cytokines, histamine, and proteases which result in allergic symptoms [7▪▪].

Immunological tolerance is an active process of immune responses to specific antigens [32]. The development of allergen tolerance is often associated with an increase in allergen-specific regulatory T (Treg), regulatory B (Breg) cells, allergen-specific IgG4 antibody production, and decreased activation of effector cells, such as basophils, mast cells, and eosinophils. Treg and Breg cells are considered as the major instrumental cells that have immunosuppressive effects [33,34]. The effects of IL-10 on monocytes and macrophages include inhibition of the production and release of mediators and antigen-presentation and the enhancement of phagocytosis [35]. IL-10 directly acts on $CD4^+$ T cells and downregulates IL-2 and interferon gamma-γ production by Th1 cells and of IL-4 and IL-5 production by Th2 cells [36]. The induction of type 2 immune response that consists of Th2 cells and type 2 innate

lymphoid cells (ILC2) together with the production of allergen-specific IgE antibodies and increased eosinophil numbers in the affected tissues and peripheral blood is a cause of allergic diseases [37–39]. The overview of the mechanism of AIT is explained in Fig. 1. These allergic symptoms have often reduced the quality of human life on a daily basis [40,41].

For several decades, the underlying mechanism of AIT has been intensively studied to understand the complexity of allergen-specific immune responses at both molecular and cellular levels [42,43]. In brief, molecular and cellular events that occur during AIT can be separated into 4 stages. Within a few hours of allergen exposure, there is a decrease in mast cell and basophil activity and degranulation. This stage is called early desensitization. Then, within a few days, allergen-specific Treg and Breg cells are generated, which leads to the suppression of allergen-specific effector T-cell subsets are generated. After a few weeks to months, the allergen-specific antibodies IgE-to-IgG4 ratio decreases substantially. Finally, decreases in tissue mast cells, eosinophils, and release of their mediators are observed after several months after AIT [28]. Interestingly, allergen-specific Treg and Breg cells and their microenvironmental cytokines are important for determining success or failure in immunotherapy AIT [30,44].

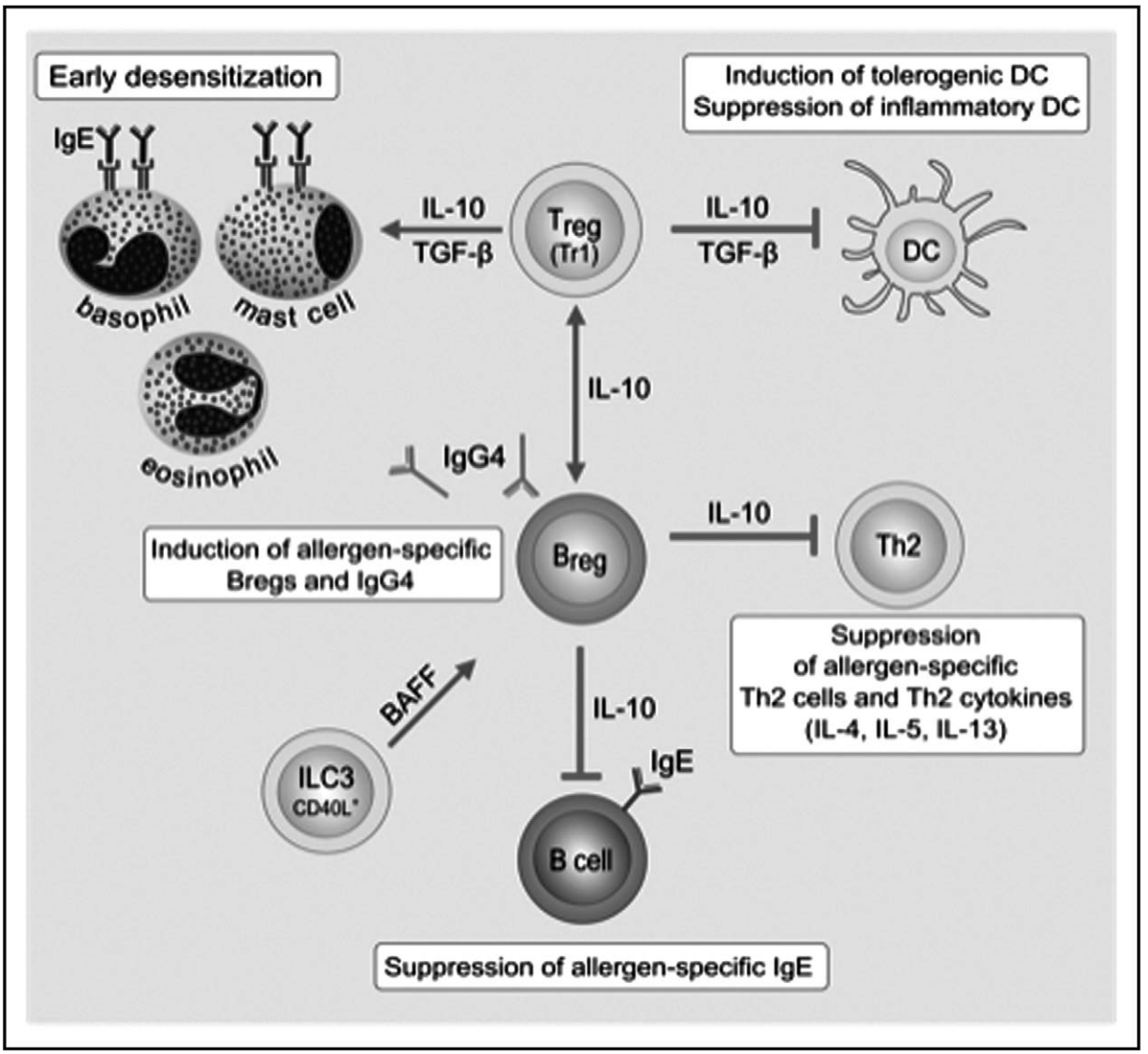

FIGURE 1. The development of allergen tolerance in AIT. *AIT*, Allergen-specific immunotherapy; *Breg*, regulatory B cells; *ILC2*, group 2 innate lymphoid cells; *ILC3*, group 3 innate lymphoid cells; *TGF-β*, transforming growth factor-β; *Th2*, T-helper 2 cells; *Tr1*, allergen-specific regulatory T cells; *Treg*, regulatory T cells.

THE INDUCTION AND DEVELOPMENT OF REGULATORY B CELLS

Komlósi *et al.* [45▪] demonstrated that activated CD40 ligand (CD40L)-expressing type 3 innate lymphoid cells (ILC3s) play an important role in Breg cell induction in human tonsils. These human circulating ILC3s were able to differentiate into the activated form of ILC3s ($CD40L^+$ ILC3s) in the presence of IL-15. Tonsillar epithelial cells and Myeloid Dendritic cells contribute to the supporting microenvironment of $CD40L^+$ ILC3s in the tissue through their IL-15 production. Naive B cells upregulated IL-15 expression on interaction with ILC3s, and $CD40L^+$ ILC3-induced IL-15 production was mediated by B cell activating factor (BAFF) in a BAFF receptor-dependent manner. Then, $CD40L^+$ ILC3s induce the development of IL-10–secreting, programmed death-ligand 1-expressing functional Breg cells.

In addition, IL-10 cytokine that plays essential roles as an effector or regulatory molecules in both innate and adaptive immune responses is secreted not only in B cells [34]. The generation of IL-10 and TGF-β-producing allergen-specific regulatory T cells (Tr1) have a potential to inhibit Th2-type responses and suppress other effector T cells through multiple mechanisms engaging cytotoxic T-lymphocyte-associated protein 4, programmed death-1 and histamine receptor 2 [46]. During AIT, IL-10 is primarily produced by allergen-specific Treg cells [47▪], subsequently by allergen-specific Breg cells (Br1 cells), and monocytes. In bee venom-allergic patients, these IL-10-secreting Tr1 cells are substantially increased very early after the start of bee venom AIT and persist in high-dose exposure to allergen models, such as nonallergic beekeepers and cat owners [48–51]. Tr1 cells are also generated in response to other immunotherapies such as sublingual immunotherapy, immunotherapy against other allergens such as grass pollen and house dust mites, and peptide immunotherapies in allergy and autoimmune diseases [52,53,54▪▪]. The immunosuppressive properties of Treg cells are also regulated by IL-10 producing Tr1 cells and $CD4^+CD25^+$ Treg cells [55]. Moreover, IL-10 together with TGF-β revealed the inhibitory functions through the other subset of Treg cells, inducible T cell co-stimulator $(ICOS)^+$ forkhead box $P3^+$ Treg cells [56]. IL-10 suppresses T cells via CD28 and ICOS-dependent T-cell costimulation by precisely blocking proliferation and cytokines production [57]. In a mice model, the transfer of ovalbumin peptide-specific $CD4^+$ $CD25^+$ T cells to OVA-sensitized mice was determined to be IL-10 dependent. Kearley *et al.* [58] demonstrated that the transfer of $CD4^+$ $CD25^+$ T cells leads to an increased expression of IL-10 in the lungs and to a decrease in airway hyperreactivity and the recruitment of eosinophils and Th2 cytokines to airways after an allergen challenge. These effects were reversed by administration of an anti-IL-10R antibody [58]. SLIT with systemic administration of IL-2 associated with an anti-IL-2 monoclonal antibody (IL-2/anti-IL-2Ab complex or IL-2C) rendered Treg-mediated tolerance and reversed the IgE-mediated food allergy in mice [59].

Three different $IL\text{-}10^+$ Breg subsets have been identified in humans. $CD19^+CD5^+CD1d^+$ and $CD19^+CD24^+CD38^+$ Bregs were shown to suppress Th1 cells [60,61], whereas $CD19^+CD25^+CD71^+CD73^-$-inducible Breg cells may play a role in allergen-specific immune tolerance [62▪▪]. These inducible Breg cells (or Br1 cells) are immunosuppressive B cells that regulate excessive inflammation via the release of IL-10, which induce differentiation of regulatory T cells and inhibit proinflammatory responses and augment IgG4 production [7▪▪,63▪▪,64▪▪,65▪,66].

Our group investigated the role of B cells in AIT, by comparing B-cell responses in allergic patients before and during AIT and naturally exposed healthy beekeepers before and during the beekeeping season. Br1 cells are characterized by high expression of CD25 and CD71 and low expression of CD73 on the cell surface. We demonstrated that phospholipase A2 (PLA)-specific B cells showed similar responses in allergic patients and beekeepers after venom exposure. Both groups showed increased frequencies of plasmablasts, PLA-specific memory B cells, and IL-10-secreting $CD73^-CD25^+CD71^+$ Br1 cells. Additionally, PLA-specific IgG4-switched memory B cells expanded after bee venom exposure [67]. The changes in the immune response induced upon venom immunotherapy have shown that 3–4 months after the start of the treatment, the frequency of PLA-specific IL-10-producing Br1 cells increased two-fold to five-fold, reaching a level comparable to that of healthy beekeepers during the season [68–70]. Interestingly, PLA-specific B cells showed increased CCR5 expression after high-dose allergen exposure, whereas CXCR4, CXCR5, CCR6, and CCR7 expression remained unaffected [71▪▪]. The prominent immunoregulatory profile of IL10-producing Breg cells characterized by upregulation of CD25 (IL-2 receptor a chain), programmed death-ligand 1, suppressor of cytokine signaling 3 (SOCS3), and glycoprotein A repetitions predominant has been demonstrated by Stanic *et al.* [72▪▪]. On a long-term follow-up of allergen-specific B cells during AIT in house dust mite allergy, we were able to show that $IgG4^+$ and immunglobulin A $(IgA)^+$ Der p 1-specific B cells

Table 1. The list of surface markers on Breg cells and Br1 cells

Types	Human	Mouse	Function
Breg cells [60,83]	$CD19^+CD5^+CD1d^+$ and $CD19^+CD24^+CD38^+$	$CD19^+CD1d^{hi}CD5^+$ and $CD19^+CD9^+$	Suppress Th2 cells, and Th2 cytokines
Br1 cells [67,84]	$CD19^+CD73^-CD25^+CD71^+$	$CD19^+CD5^+$	Suppress IgE production, upregulate of IgG4 production in human, and increase IL-10 production both in human and mouse
Immature B cells [61,85–87]	$CD19^+CD24^{hi}CD38^{hi}$	$CD19^+CD21^{hi}CD23^{hi}$ $CD24^{hi}IgM^{hi}IgD^{hi}CD1d^{hi}$	Transitional B cells based on the phenotypes and ontogeny
Plasmablasts [88]	$CD27^{int}CD38^{hi}$	$CD138^+CD44^{hi}$	A short-lived and proliferating antibody-secreting cells

Breg, regulatory B cells; *Br1*, allergen-specific Breg cells; *Th2*, T-helper 2 cells.

showed a significant increase after AIT. Responders showed a significantly greater frequency compared with nonresponders in the IgG4 but not the IgA fraction. The frequency of plasmablasts and $IL\text{-}10^+$ and/or $IL\text{-}1RA^+$ producing Breg cells was greater among responders compared with nonresponders after 2 years. The increased frequency of Der p 1-specific $IgG4^+$ B cells, plasmablasts, and $IL\text{-}10^+$ and dual-positive $IL\text{-}10^+IL\text{-}1RA^+$ Breg cells significantly correlated with improved clinical symptoms over the course of AIT. Therefore, allergen-specific B cells in patients responding to AIT are characterized by increased numbers of IgA^+ and $IgG4^+$expressing Der p 1-specific B cells, plasmablasts, and $IL\text{-}10^+$ and/or $IL\text{-}1RA^+$ Breg cells [73▪▪]. In asthmatic mice, the adoptive transfer of CD9(+) B cells normalized airway inflammation and lung function by inhibiting Th2 and Th17-driven inflammation in an IL-10-dependent manner, restoring a favorable immunological balance in lung tissues. They further showed that injection of $CD9^+$ Breg cells controls the expansion of lung effector T cells allowing the establishment of a favorable regulatory T cells/effector T cells ratio in lungs [74]. Several surface markers on Breg cells are comprehensively described in a recent review article [64▪▪]. Here, we provide a list of surface markers on different B-cell subsets in both human and mouse in Table 1.

Additionally, allergen-specific IgE which is produced by B cells indisputably plays a key role in determining the allergen specificity of allergic disease [75]. Characterization of B-cell receptor repertoires in different tissues and the evolution of IgE repertoires in allergen-specific B cells has become an interesting topic to enhance the understanding of allergic responses [76]. A recent study demonstrated that Ara h 2-specific circulating memory B cells are induced early and transiently in patients undergoing peanut oral immunotherapy and can be identified by using a fluorescent multimer. Immunoglobulins from these circulating Ara h 2-specific B cells are affinity matured and some clonal groups are shared among unrelated patients with peanut allergy [77]. Hoh *et al.* [78] showed peanut allergen-specific B cells in patients express mutated antibody genes, usually of switched isotypes and bind to both linear and conformational epitopes. Even well-defined linear epitopes of allergen proteins can be recognized by multiple independent B-cell clones in a single patient. Increased frequencies of allergen-binding B cells, progressive somatic mutation of IgG4, but not IgE, are observed during AIT [78]. Therefore, IgE repertoire persistence and evolution could be considered as markers for monitoring AIT in patients with allergic diseases [79].

Together these studies provide a first detailed characterization of allergen-specific B cells before and after immune tolerance induction in bee venom, house dust mite, and peanut allergies as shown in Table 2. It is clear that the responses of B cells in AIT are very essential; however, to get a full understanding of all mechanisms, they need to be further investigated in-depth.

CONCLUSION

AIT has been considered as the most powerful treatment in patients who suffer from allergy, cancer, and autoimmune diseases [13,80]. Immune tolerance development was aimed for allergy and autoimmunity treatment, whereas enhancement of immune effector functions was aimed in cancer therapy. The development of immune tolerance requires allergen-specific Breg and Treg cells, increased IgG4 isotype-specific antibody response, and decreased activation of effector cells, such as basophils, mast cells, and eosinophils. Breg cells are interesting targets for the improvement of new therapies in the induction of immune tolerance. Advances in mechanisms of AIT has been aiming

Table 2. B cells and antibody responses in the induction of allergen-specific immune tolerance

Disease model	Types	Changes in the response to tolerant VS allergic state	References
Bee venom allergy	Expansion of Br1 cells	Increased frequencies of plasmablasts, PLA-specific memory B cells, and IL-10-secreting $CD73^{-}CD25^{+}CD71^{+}$ Br1 cells. PLA-specific IgG4-switched memory B cells expanded after bee venom exposure Increased CCR5 expression after high-dose allergen exposure, whereas CXCR4, CXCR5, CCR6, and CCR7 expression remained unaffected	[67,71▪▪]
House dust mite allergy	Expansion of Br1 cells	Increased numbers of IgA and IgG4-expressing Der p 1-specific B cells, plasmablasts, and $IL\text{-}10^{+}$ and/or $IL\text{-}1RA^{+}$ Breg cells	[73▪▪]
Peanut allergy	Allergen-specific antibodies	Increasing somatic mutation of IgG4 B cells clones, whereas IgE mutation levels in the clone did not increase Ara h 2-specific B-cell receptor repertoire is oligoclonal and somatically hypermutated and shares similar clonal groups in AIT The allergen-specific B cells clones had increased persistence, higher likelihood of belonging to clones expressing other switched isotypes, and possibly larger clone size	[77,78,79]

AIT, Allergen-specific immunotherapy; *Breg*, regulatory B cells; *Br1*, allergen-specific Breg cells; *PLA*, phospholipase A2.

to ensure that more efficient AIT vaccines are developed with low side-effects and higher efficacy and allergic patients will receive precise medical treatments [81,82].

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
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